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Sperm characters of the digenean *Prosorhynchus aculeatus* Odhner, 1905 (Bucephalidae), a parasite of the marine fish *Conger conger* (Linnaeus, 1758) (Congridae)

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Abstract

Within the Digenea, the family Bucephalidae includes numerous species parasitizing mainly marine and freshwater fishes. This family includes five recognized subfamilies, and ultrastructural data on their sperm cells are very scarce. The existing data are restricted to the subfamily Bucephalinae. Thus, the present study is the first complete analysis of the sperm

cell of a bucephalid belonging to the subfamily Prosorhynchinae. Herein, we describe the ultrastructure of the mature spermatozoon of *Prosorhynchus aculeatus*, a parasite of the conger eel *Conger conger*, assessed by means of transmission electron microscopy (TEM). The spermatozoon of *P. aculeatus* is a filiform cell that presents two axonemes of the 9+'1' pattern of trepaxonematan Platyhelminthes, parallel cortical microtubules, mitochondrion, nucleus, external ornamentation of the plasma membrane, spine-like bodies and a large amount of glycogen granules. According to the anterior location of the external ornamentation of the plasma membrane, *P. aculeatus* presents a Quilichini et al.'s type 1 spermatozoon. With respect to the posterior extremity, the sperm cell of *P. aculeatus* corresponds to the Quilichini et al.'s cryptogonimidean type. Our results are compared with those of the two previously studied bucephalids (Bucephalinae), *Prosorhynchoides gracilescens* and *Pseudorhipidocotyle elopichthys*.

Key words: *Prosorhynchus aculeatus*, Bucephalidae, Digenea, sperm characters, ultrastructure

Introduction

The study of sperm characters has been proven as a useful tool in the analysis of relationships of Platyhelminthes, particularly in the case of monogeneans and cestodes (Justine 1991a, b, 1995, 1998, 2001; Bâ and Marchand 1995; Levron et al. 2010). With respect to the Digenea, some authors have attempted to analyse the potential usefulness of diverse ultrastructural characteristics of sperm cells (see Quilichini et al. 2010, 2011; Bakhoum 2012). However, ultrastructural descriptions of sperm cell characters are still missing concerning many digenean families, such as the Bucephalidae. In fact, the available information on the Bucephalidae concerns only two species of the subfamily Bucephalinae: *Prosorhynchoides*

gracilescens and *Pseudorhipidocotyle elopichthys* (Erwin and Halton 1983; Tang et al. 1998). Furthermore, the study on *P. gracilescens* (published as *Bucephaloides gracilescens*) contains several misinterpretations of TEM micrographs and the study on *P. elopichthys* presents a very scarce amount of data.

The Bucephalidae is a complex family, which includes about 25 genera and 380 species parasitizing the swim-bladder, the body cavity, the stomach or the intestine of mainly marine and freshwater fishes (Nolan et al. 2015). This family was formerly known as Gasterostomatidae, but the genus *Gasterostomum* was synonymized with *Bucephalus* and thus Bucephalidae was retained as the correct family name. The Bucephalidae includes five subfamilies, namely the Bucephalinae, the Dolichoenterinae, the Heterobucephalopsinae, the Paurorhynchinae and the Prosorhynchinae (see Overstreet and Curran 2002; Nolan et al. 2015). According to the recent combined analysis of morphology and genetic data, the subfamily Heterobucephalopsinae is basal to the remaining four subfamilies. The Dolichoenterinae and the Prosorhynchinae are monophyletic sister clades, basal to the Bucephalinae and to the Paurorhynchinae. Contrarily, bucephalines appear as a paraphyletic group (see Nolan et al. 2015). Within the subfamily Prosorhynchinae there is the cosmopolitan genus *Prosorhynchus*, including species parasitizing the intestine of marine fishes (see Overstreet and Curran 2002).

In order to increase datasets on the ultrastructural organization of spermatozoa characters in the Bucephalidae, the present study provides information on *Prosorhynchus aculeatus* (Prosorhynchinae). Together with future ultrastructural studies on the spermatozoon of bucephalids, the present results will contribute to a better understanding of relationships within this digenean family.

Materials and methods

Live adult specimens of *Prosorhynchus aculeatus* were collected during May 2013 from the digestive tract of several conger eels *Conger conger* (Linnaeus, 1758) (Pisces: Congridae) captured in the Mediterranean Sea, off Palamós (Girona, Spain).

Several worms were rinsed with a 0.9% NaCl solution and fixed in cold (4 °C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4 for a minimum of 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in cold (4 °C) 1% osmium tetroxide with 0.9% potassium ferricyanide [$K_3Fe(CN)_6$] in the same buffer for 1 h, rinsed in Milli-Q water (Millipore Gradient A10), dehydrated in an ethanol series and propylene oxide, embedded in Spurr's resin and polymerized at 60 °C for 72 h. Ultrathin sections (60–90 nm thick) of the seminal vesicle were obtained using a Reichert-Jung Ultracut E ultramicrotome. Sections were placed on 200-mesh copper and gold grids. Sections placed on copper grids were double-stained with uranyl acetate and lead citrate according to the Reynolds (1963) procedure. Copper grids were examined in a JEOL 1010 transmission electron microscope operated at an accelerating voltage of 80 kV, in the "Centres Científics i Tecnològics" of the University of Barcelona (CCiTUB).

Sections placed on gold grids were treated according to the Thiéry (1967) test to reveal the presence of glycogen. Thus, they were treated in periodic acid (PA), thiocarbohydrazide (TCH) and silver proteinate (SP) as follows: 30 min in 10% PA, rinsed in MilliQ water, 24 h in TCH, rinsed in acetic solutions and MilliQ water, 30 min in 1% SP in the dark, and rinsed in MilliQ water. Sections were examined in a JEOL 1010 transmission electron microscope in the CCiTUB.

Results

The mature spermatozoon of *Prosorhynchus aculeatus* is a filiform cell exhibiting the main characteristics of digenean spermatozoa: two axonemes of the 9+'1' pattern of

trepaxonematan Platyhelminthes, parallel cortical microtubules, mitochondrion, nucleus, external ornamentation of the plasma membrane, spine-like bodies and large amount of glycogen granules. The organization and location of all these characters along the male gamete permit us to establish three regions (I to III) from the anterior to the posterior sperm extremities (Figs. 1 to 4).

Region I (Figs. 1a–g and 4I) corresponds to the anterior region of the spermatozoon. This region is mainly characterized by the presence of external ornamentation of the plasma membrane (Figs. 1a–g and 4I), surrounding nearly all the perimeter of the sperm cell, progressively disappearing toward the posterior area of region I (Fig. 1g). In fact, the complete disappearance of the external ornamentation marks the transition between regions I and II. Spine-like bodies are also present along region I (Figs. 1c, e–g and 4I). The two axonemes are longitudinally displaced in relation to one another (Figs. 1b–e and 4I). Cortical microtubules are parallel to the hypothetical longitudinal sperm axis and they consist in a submembraneous and continuous layer that totally surrounds the cell in the anterior area of region I (Figs. 1a–d and 4I), while in more posterior area they are discontinuous (Figs. 1e–g and 4I).

Region II (Figs. 1h–l, 3 and 4II) corresponds to the middle region of the spermatozoon. The external ornamentation of the plasma membrane and spine-like bodies are no longer present in the anterior part of region II. This region is characterized by the presence of the two axonemes, by the progressive organization of cortical microtubules into two bundles (Fig. 1h, i, k, l) and by the appearance of the mitochondrion in the posterior area (Figs. 1l and 4II). A large amount of granular material also appears along this region (Figs. 1j–l). The glycogenic nature of this granular material has been determined by the Thiéry test (Fig. 3). Notice the presence of glycogen between the central core and peripheral doublets of axonemes (Fig. 1j–l).

Region III (Figs. 2a–j, 3 and 4III) corresponds to the nuclear and posterior extremity of the spermatozoon. The presence of the nucleus is the main characteristic of this region (Figs. 2a–h and 4III). As in region II, glycogen is present between the central core and the peripheral doublets of the axonemes in the anterior areas of region III (Figs. 2a and 3). Along region III, the nucleus progressively increases in size as the mitochondrion disappears (Fig. 2a–e). The posterior extremity of the sperm cell is marked by (i) the disorganization of one axoneme (Figs. 2d and 4III), followed by (ii) the disappearance of the mitochondrion (Figs. 2f and 4III), and by (iii) the disorganization of the other axoneme (Figs. 1g and 4III). During the progressive disorganization of the second axoneme there is a reduction in nucleus size (Fig. 2g, h) and in the number of cortical microtubules (Fig. 2g–i). Thus, the posterior spermatozoon tip is characterized by the presence of doublets and singlets of the second axoneme (Fig. 2j).

Discussion

The mature spermatozoon of *Prosorhynchus aculeatus* is a filiform cell, which exhibits a similar ultrastructural organization to that reported for most digenean species. However, the present study allowed describing the particular arrangement of several characters in all 3 regions of the sperm cell.

Anterior spermatozoon region

The anterior region of the spermatozoon of digeneans shows a number of characters potentially useful for phylogenetic inference (see Quilichini et al. 2011; Bakhoum et al. 2017).

The character of external ornamentation of the plasma membrane is usually present in the spermatozoon of digeneans. According to Quilichini et al. (2011), there are three groups of

digenean spermatozoa defined according to the presence/absence and location of the external ornamentation of the plasma membrane. Thus, a first group is characterized by the presence of the external ornamentation in the proximal area of the sperm cell, a second group exhibits the ornamentation in a more distal area of the gamete, and finally a third group includes spermatozoa that lack ornamentation. The sperm cell of *P. aculeatus* is clearly included in the first group, presenting the external ornamentation of the plasma membrane in a large area of the anterior spermatozoon extremity. The other studies on the ultrastructure of bucephalid spermatozoa (*Prosorhynchoides gracilescens* and *Pseudorhipidocotyle elopichthys* -Erwin and Halton 1983; Tang et al. 1998), present cross-sections exhibiting external ornamentation associated only with cortical microtubules, which compares well with the organization observed in the sperm cell of *P. aculeatus* in the present study. We suggest that the ornamented sections described in *P. gracilescens* and *P. elopichthys* (Erwin and Halton 1983; Tang et al. 1998) correspond to anterior areas of the gamete.

Spine-like bodies, which are usually associated with the external ornamentation of the plasma membrane, were described for the first time in the opecoelid *Opecoeloides furcatus* (Miquel et al. 2000). Since then, spine-like bodies have been generally described in many studies on the ultrastructure of spermatozoa. These structures can be observed in TEM micrographs of older works on male gametes, but authors failed to mention their presence and probably considered them to be artefacts (Justine and Mattei 1982; Orido 1988). In our opinion, at the present state of knowledge provided by the ultrastructural studies of the spermatozoon in bucephalids, no conclusion can be drawn concerning spine-like bodies. Thus, the absence of spine-like bodies in previous studies on bucephalids (*P. gracilescens* and *P. elopichthys* -Erwin and Halton 1983; Tang et al. 1998) and its presence in *P. aculeatus* highlight the need for future ultrastructural works, particularly on this character, in species belonging to the Bucephalidae.

Mitochondrial region

As in all digeneans, the spermatozoon of *P. aculeatus* presents a mitochondrion. There are different viewpoints concerning the number of mitochondria present in the mature spermatozoon of digeneans. According to Burton (1972), during spermiogenesis, several mitochondria are present in the zone of differentiation, penetrate into the spermatid body and fuse to form a long mitochondrion. Indeed, most of the ultrastructural studies of digeneans describe the presence of a single mitochondrion in the male gamete. However, several authors have considered the presence of two mitochondria, and even three (see Bakhoum 2012 for a review). In the spermatozoon of *P. aculeatus* there is only one mitochondrion and for the remaining bucephalids studied until now, *P. gracilescens* and *P. elopichthys*, TEM micrographs seem to show a similar situation (Erwin and Halton 1983; Tang et al. 1998). As in most digeneans, including *P. aculeatus*, when a single mitochondrion is present, it is located in the middle region of the sperm cell and it usually overlaps the anterior part of the nucleus.

Posterior spermatozoon region

Several authors have considered the nucleus as a typical character of the anterior region. This is the case of the studies on the two bucephalids studied until now, *P. gracilescens* and *P. elopichthys* (Erwin and Halton 1983; Tang et al. 1998). However, the fertilization study in the digenean *Gonapodasmius* sp. (Justine and Mattei 1984) determined that the extremity of the spermatozoon containing the nucleus is the last region of the male gamete to fuse with the oocyte. Since then, it has been universally accepted that the region containing the nucleus is the posterior extremity of the sperm cell.

With respect to the posterior region of digenean spermatozoa, the disappearing sequence of the principal characters (second axoneme, cortical microtubules and nucleus) toward the posterior spermatozoon tip is another interesting aspect. Quilichini et al. (2010) consider three types of posterior spermatozoon extremities in the Digenea. A first type (opecoelidean) is characterised by the sequence 'posterior axonemal extremity-posterior nuclear extremity-cortical microtubules'. A second type (fasciolidean) is characterised by the sequence 'cortical microtubules-posterior axonemal extremity-posterior nuclear extremity'. Finally, a third type (cryptogonimidean) is characterised by the sequence 'cortical microtubules-posterior nuclear extremity-posterior axonemal extremity'. The posterior spermatozoon extremity of *P. aculeatus* seems to correspond to the cryptogonimidean type, even though cortical microtubules are still present when the nucleus is no longer observed. A similar situation is observed in the two studied bucephalids *P. gracilescens* and *P. elopichthys* (Erwin and Halton 1983; Tang et al. 1998), for which authors show TEM micrographs of the posterior spermatozoon extremity sections with a single axoneme and few cortical microtubules.

Considering that some species do not completely fit the character disappearing sequences described by Quilichini et al. (2010), other authors have recently proposed the use of only the posterior spermatozoon character to identify spermatozoon posterior extremity types (Bakhoum et al. 2017). The use of only one character avoids the existing discrepancies for some species in the transition of characters toward the posterior spermatozoon extremity. Thus, the cryptogonimidean type of posterior sperm extremity should consider only the axoneme as the terminal character. In fact, the presently available ultrastructural data on the spermatozoon of three bucephalid genera (*Prosorhynchus*, *Prosorhynchoides* and *Pseudorhipidocotyle*) indicate the cryptogonimidean type as characteristic for bucephalids. This is also the case of numerous species included in other taxa (for a review see Bakhoum 2012). Among these taxa, we emphasise the families Hemiuridae with studies on four species

from three different genera (Ndiaye et al. 2012, 2013, 2014) and Pleurogenidae with four studied genera (Miquel et al. 2013; Bruňanská et al. 2014).

Concluding remarks

The ultrastructural organization of the mature spermatozoon in bucephalids corresponds to the classical pattern found in most digeneans. However, some characters should be highlighted in this family, including (1) the presence of the external ornamentation in the anterior region of the spermatozoon, (2) the presence of a single mitochondrion and (3) the second axoneme as the most terminal character of the sperm cell. Nevertheless, these facts should be considered with caution due to the scarce ultrastructural data on bucephalids. Also, other characters such as the spine-like bodies should be carefully studied in future works on the Bucephalidae.

Acknowledgements

The authors are grateful to the fishermen Mr. Miquel Mir and Mr. Francesc Subirats. We are also grateful to 'Centres Científics i Tecnològics' of the University of Barcelona (CCiTUB) for her assistance in the preparation of samples.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bâ CT, Marchand B (1995) Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. Mém Mus Natn Hist Nat Paris 166:87–95

Bakhoum AJS (2012) Contribution à la connaissance de l'ultrastructure de la spermiogenèse et du spermatozoïde des Digènes. PhD Thesis, University of Barcelona, 284 pp. http://www.tdx.cat/handle/10803/109050

Bakhoum AJS, Kacem H, Neifar L, Miquel J (2017) The Opecoelidae sperm model and its contribution to phylogeny: Spermatozoon ultrastructural particularities of *Allopodocotyle pedicellata* (Plagioporinae, Digenea, Platyhelminthes). Zool Anz 266:28–34. doi: 10.1016/j.jcz.2016.10.006

Bruňanská M, Brázová T, Zhokov AE, Poddubnaya LG (2014) Ultrastructural features of the spermatozoon and its differentiation in *Brandesia turgida* (Brandes, 1888) (Digenea, Microphalloidea, Pleurogenidae). Parasitol Res 113:2483–2491. doi: 10.1007/s00436-014-3897-6

Burton PR (1972) Fine structure of the reproductive system of a frog lung-fluke. III. The spermatozoon and its differentiation. J Parasitol 58:68–83

Erwin BE, Halton DW (1983) Fine structural observations on spermatogenesis in a progenetic trematode, *Bucephaloides gracilescens*. Int J Parasitol 13:413–426. doi: 10.1016/S0020-7519(83)80003-4

Justine J-L (1991a) Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. Can J Zool 69:1421–1440. doi: 10.1139/z91-203

Justine J-L (1991b) Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. Int J Parasitol 21:821–838. doi: 10.1016/0020-7519(91)90151-V

Justine J-L (1995) Spermatozoal ultrastructure and phylogeny of the parasitic Platyhelminthes. Mém Mus Natn Hist Nat Paris 166:55–86

Justine J-L (1998) Spermatozoa as phylogenetic characters for the Eucestoda. J Parasitol 84:385–408. doi: 10.2307/3284502

Justine J-L (2001) Spermatozoa as phylogenetic characters for the Platyhelminthes. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor and Francis, London, pp 231–238

Justine J-L, Mattei X (1982) Réinvestigation de l'ultrastructure du spermatozoïde d'*Haematoloechus* (Trematoda: Haematoloechidae). J Ultrastruct Res 81:322–332

Justine J-L, Mattei X (1984) Ultrastructural observations on the spermatozoon, ovocyte and fertilization process in *Gonapodasmius*, a gonochoristic Trematode (Trematoda: Digenea: Didymozoidae). Acta Zool (Stockh) 65:171–177. doi: 10.1111/j.1463-6395.1984.tb00822.x

Levron C, Miquel J, Oros M, Scholz T (2010) Spermatozoa of tapeworms (Platyhelminthes, Eucestoda): advances in ultrastructural and phylogenetic studies. Biol Rev 85:523–543. doi: 10.1111/j.1469-185X.2009.00114.x

Miquel J, Nourrisson C, Marchand B (2000) Ultrastructure of spermiogenesis and the spermatozoon of *Opecoeloides furcatus* (Trematoda, Digenea, Opecoelidae), a parasite of *Mullus barbatus* (Pisces, Teleostei). Parasitol Res 86:301–310. doi: 10.1007/s004360050047

Miquel J, Vilavella D, Świderski Z, Shimalov VV, Torres J (2013) Spermatological characteristics of Pleurogenidae (Digenea) inferred from the ultrastructural study of *Pleurogenes claviger*, *Pleurogenoides medians* and *Prosotocus confusus.* Parasite 20:28. doi: 10.1051/parasite/2013028

Ndiaye PI, Diagne PM, Sène A, Bakhoum AJS, Miquel J (2012) Ultrastructure of the spermatozoon of the digenean *Lecithocladium excisum* (Rudolphi, 1819) (Hemiuroidea:

Hemiuridae), a parasite of marine teleosts in Senegal. Folia Parasitol 59:173–178. doi: 10.14411/fp.2012.024

Ndiaye PI, Bakhoum AJS, Sène A, Miquel J (2013) Ultrastructure of the spermatozoon of *Parahemiurus merus* (Linton, 1910) (Digenea: Hemiuroidea: Hemiuridae), a parasite of *Sardinella aurita* Valenciennes, 1847 and *S. maderensis* (Lowe, 1838) (Teleostei: Clupeidae) in the Senegalese coast. Zool Anz 252:572–578. doi: 10.1016/j.jcz.2012.11.005

Ndiaye PI, Quilichini Y, Sène A, Tkach VV, Bâ CT, Marchand B (2014) Ultrastructural characters of the spermatozoa in Digeneans of the genus *Lecithochirium* Lühe, 1901 (Digenea, Hemiuridae), parasites of fishes: comparative study of *L. microstomum* and *L. musculus*. Parasite 21:49. doi: 10.1051/parasite/2014050

Nolan MJ, Curran SS, Miller TL, Cutmore SC, Cantacessi C, Cribb TH (2015) *Dollfustrema durum* n. sp. and *Heterobucephalopsis perardua* n. sp. (Digenea: Bucephalidae) from the giant moray eel, *Gymnothorax javanicus* (Bleeker) (Anguilliformes: Muraenidae), and proposal of the Heterobucephalopsinae n. subfam. Parasitol Int 64:559–570. doi: 10.1016/j.parint.2015.07.003

Orido Y (1988) Ultrastructure of spermatozoa of the lung fluke, *Paragonimus ohirai* (Trematoda: Troglotrematidae), in the seminal receptacle. J Morphol 196:333–343.

Overstreet RM, Curran SS (2002) Superfamily Bucephaloidea Poche, 1907. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, Vol. 1. CAB International and The Natural History Museum, London, pp 67–110

Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand B (2010) Ultrastructural study of the spermatozoon of *Heterolebes maculosus* (Digenea, Opistholebetidae), a parasite of the porcupinefish *Diodon hystrix* (Pisces, Teleostei). Parasitol Int 59:427–434. doi: 10.1016/j.parint.2010.06.002

Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand B (2011) Spermatozoon ultrastructure of *Gyliauchen* sp. (Digenea: Gyliauchenidae), an intestinal parasite of *Siganus fuscescens* (Pisces: Teleostei). Biol Bull 221:197–205

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Tang J, Wang W, Wang G (1998) Studies on ultrastructure of spermatogenesis and sperm in *Pseudorhipidocotyle elpichthys*. Acta Hydrobiol Sin 22:168–173

Thiéry JP (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J Microsc 6:987–1018

Legends to figures

Fig. 1 Prenuclear or anterior regions (I and II) of the spermatozoon of *Prosorhynchus aculeatus*. **(a–g)** Consecutive cross-sections of region I showing the appearance of both axonemes and the progressive reduction in the number of cortical microtubules and in the external ornamentation of the plasma membrane. **(h, i)** Cross-sections of anterior area of region II. **(j, k)** Cross- and longitudinal sections of middle area of region II characterized by the presence of glycogen. Note the particular location of glycogen between the central core and peripheral doublets of axonemes. **(l)** Cross-section of a posterior area of region II characterized by the presence of the mitochondrion. *Ax1* first axoneme, *C2* centriole of the second axoneme, *CC1* central core of the first axoneme, *CC2* central core of the second axoneme, *CM* cortical microtubules, *EO* external ornamentation of plasma membrane, *G* glycogen, *M* mitochondrion, *SB* spine-like bodies. *Scale bars* 300 nm.

Fig. 2 Nuclear or posterior region (III) of the spermatozoon of *Prosorhynchus aculeatus*. **(a–e)** Cross-sections showing the progressively increasing nucleus size. Note the disorganization

of the first axoneme in d. **(f)** Cross-section without mitochondrion. **(g, h)** Consecutive cross-sections toward the disorganization of the second axoneme. **(i, j)** Cross-sections of the posterior spermatozoon extremity containing some cortical microtubules and microtubules of the second axoneme. *CM* cortical microtubules, *D* doublets, *G* glycogen, *M* mitochondrion, *N* nucleus, *S* singlets. *Scale bars* 300 nm.

Fig. 3 Glycogen labelling by means of Thiéry test. *G* glycogen, *M* mitochondrion, *N* nucleus. *Scale bar* 300 nm.

Fig. 4 Schematic reconstruction of the spermatozoon of *Prosorhynchus aculeatus*. In order to make the diagram clearer, the granules of glycogen were omitted in the longitudinal section. *ASE* anterior spermatozoon extremity, *Ax1* first axoneme, *Ax2* second axoneme, *C1* centriole of the first axoneme, *C2* centriole of the second axoneme, *CM* cortical microtubules, *D* doublets, *EO* external ornamentation of plasma membrane, *G* glycogen, *M* mitochondrion, *N* nucleus, *PM* plasma membrane, *PSE* posterior spermatozoon extremity, *S* singlets, *SB* spine-like bodies.

Figure 1

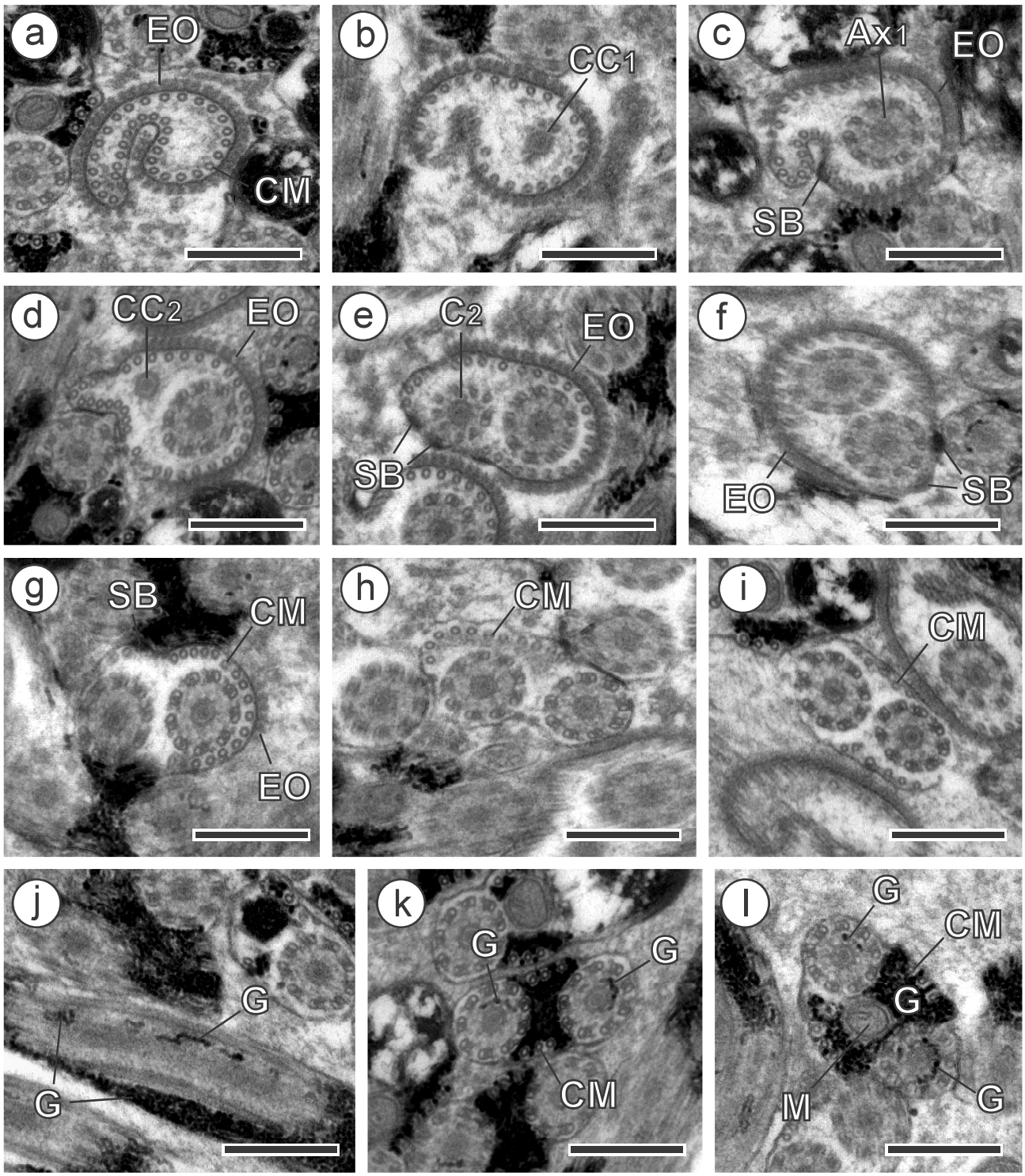
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Figure 2

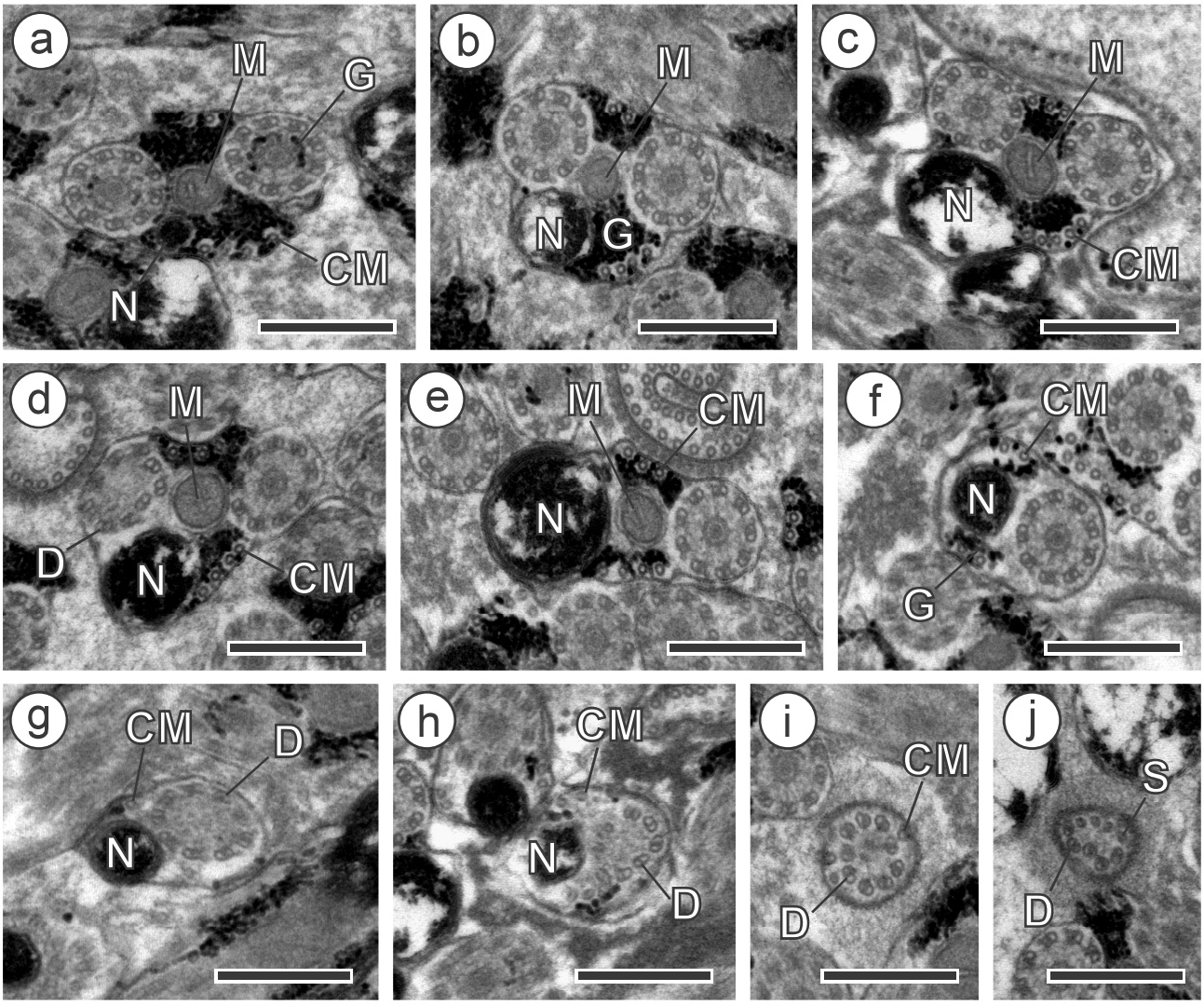


Figure 3

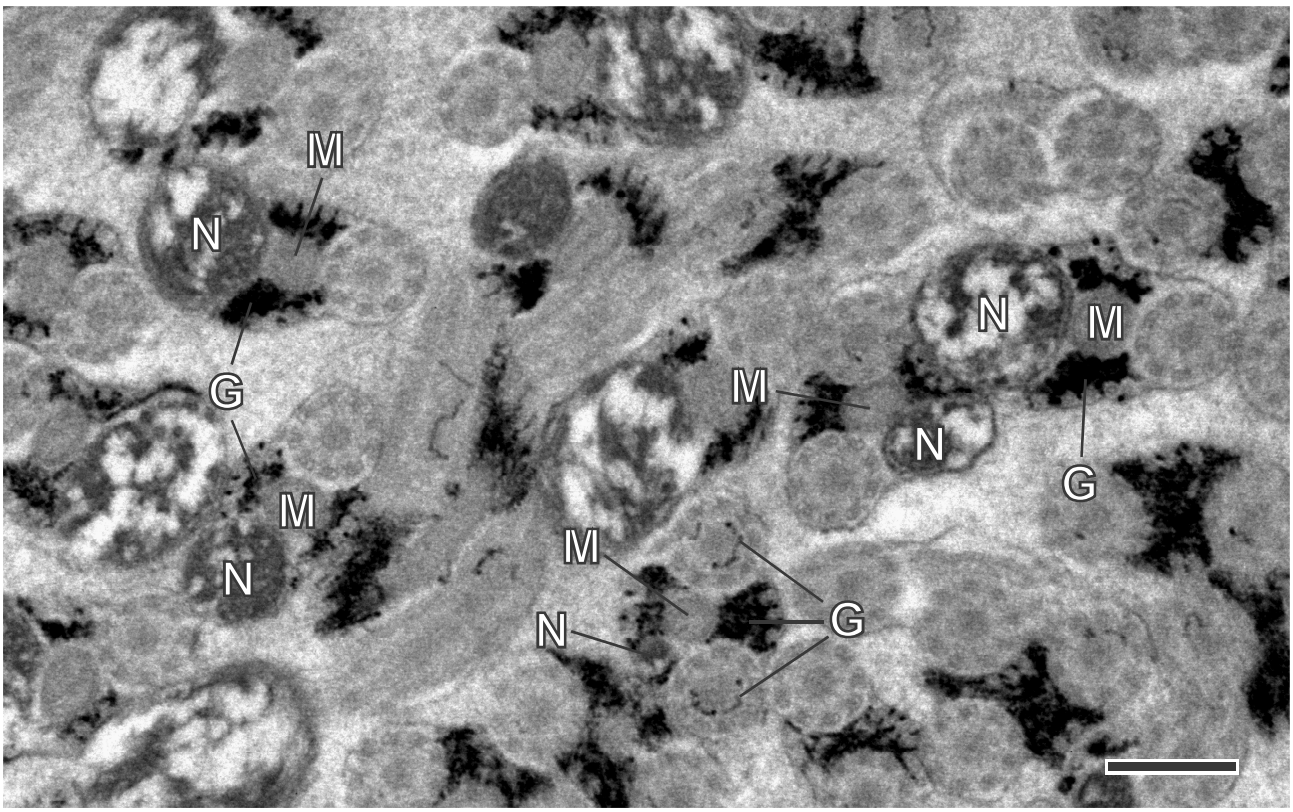
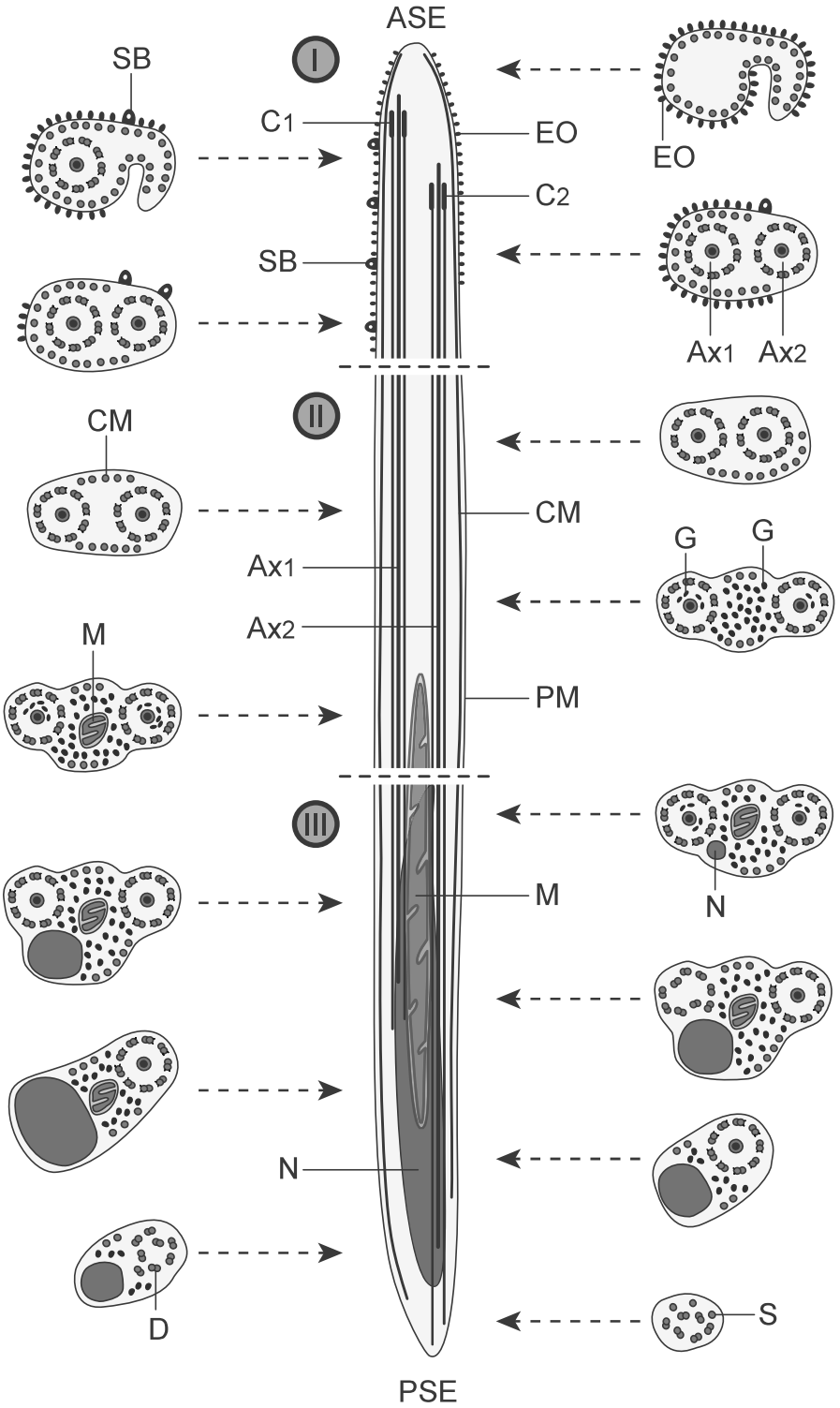


Figure 4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